Evidence for Simultaneous Abiotic−Biotic Oxidations in a Microbial-Fenton's System

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The conditions that support the simultaneous activity of hydroxyl radicals (OH•) and heterotrophic aerobic bacterial metabolism were investigated using two probe compounds: (1) tetrachloroethene (PCE) for the detection of OH• generated by an iron−nitrilotriacetic acid (Fe−NTA) catalyzed Fenton-like reaction and (2) oxalate (OA) for the detection of heterotrophic metabolism of *Xanthobacter flavus*. In the absence of the bacterium in the quasi-steady-state Fenton's system, only PCE oxidation was observed; conversely, only OA assimilation was found in non-Fenton's systems containing *X. flavus*. In combined Fenton's-microbial systems, loss of both probes was observed. PCE oxidation increased and heterotrophic assimilation of OA declined as a function of an increase in the quasi-steady-state H_2O_2 concentration. Central composite rotatable experimental designs were used to determine the conditions that provide maximum simultaneous abiotic−biotic oxidations, which were achieved with a biomass level of 10^9 CFU/mL, 4.5 mM H_2O_2, and 2.5 mM Fe−NTA. These results demonstrate that heterotrophic bacterial metabolism can occur in the presence of hydroxyl radicals. Such simultaneous abiotic−biotic oxidations may exist when H_2O_2 is injected into the subsurface as a microbial oxygen source or as a source of chemical oxidants. In addition, hybrid abiotic−biotic systems could be used for the treatment of waters containing biorefractory organic contaminants present in recycle water, cooling water, or industrial waste streams.

Introduction

In situ bioremediation has been widely used for the treatment of contaminated soils and groundwater and has been effective in destroying petroleum hydrocarbons and other reduced contaminants under aerobic conditions (*1−3*). Because in situ aerobic bioremediation is usually limited by oxygen, stabilized H_2O_2 has been injected into the subsurface to supply oxygen through its decomposition by bacterial catalase (*4*). Although this practice was originally thought to supply oxygen hundreds of meters downgradient from the source of injection, Spain et al. (*5*) showed that an excessive loss of H_2O_2 occurred in the presence of biofilms surrounding injection wells, and the use of H_2O_2 for bioremediation has since declined significantly.

More recently, H_2O_2 has been injected into the subsurface to promote the in situ chemical oxidation of organic contaminants by Fenton-like reactions. Such modified Fenton's systems are based on the standard Fenton's reaction in which the decomposition of a solution of dilute hydrogen peroxide is catalyzed by excess iron(II) at acidic pH, resulting in near-stoichiometric generation of hydroxyl radicals (OH•) (*6*):

$$H_2O_2 + Fe^{2+} \rightarrow OH^\bullet + OH^- + Fe^{3+} \qquad (1)$$
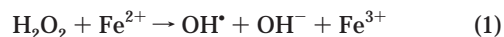
Catalyst modifications such as iron(III) salts (*7*), iron chelates at neutral pH (*8*), and naturally occurring iron minerals (*9−14*) have been used to promote the chemical oxidation of contaminants in soils and groundwater. In situ chemical oxidation using modified Fenton's reactions has gained increased attention for groundwater remediation with over 100 applications of this process (*15*).

The potential for OH•-mediated oxidations during H_2O_2-enhanced in situ bioremediation has been proposed but has never been investigated. Conversely, the potential for heterotrophic aerobic metabolism during in situ chemical oxidation using modified Fenton's reagent has never been considered. If such coexisting abiotic−biotic oxidations occur in either system, these reactions may enhance the treatment of contaminants in the subsurface and promote their mineralization. Furthermore, if these coexisting reactions occur, the process design for bioremediation or chemical oxidation may require changes to promote both abiotic and biotic processes for optimal remediation of the subsurface. The coexisting abiotic−biotic oxidation of chemical compounds could also have important implications for the design of ex situ waste treatment systems.

Documenting the occurrence of abiotic oxidations in microbial systems that receive H_2O_2 as an oxygen source or chemical oxidant is problematic. Although the presence of OH• can be measured both directly and indirectly (*16, 17*) and procedures for quantifying microbial metabolism are well established (*18−21*), evaluating the simultaneous activity of OH• and aerobic microbial metabolism is difficult because both are oxidative processes. Many compounds that are metabolized by aerobic heterotrophic metabolism (e.g., aliphatic and aromatic hydrocarbons) also react rapidly with OH•, making the two processes difficult to distinguish. The purpose of this research was to develop a probe system capable of distinguishing between OH•-mediated oxidations and heterotrophic aerobic metabolism and to use this probe system for evaluating coexisting OH• oxidations and heterotrophic aerobic metabolism in a microbial-Fenton's system.

Experimental Section

Materials. Tetrachloroethene (perchloroethylene, PCE) was purchased from Eastman Kodak. Oxalic acid (99.9%), 2-propanol (99.9%), and trichloromethane (99.9%) were obtained from J. T. Baker. Iron(III) sulfate, nitrilotriacetic acid (NTA) (98.5%), potassium hydroxide (KOH), and oxalate diagnostic kits were supplied by Sigma. Reagents used for bacterial media were also purchased from Sigma, except nutrient broth and Noble agar, which were purchased from Difco. Hexane was obtained from Fisher Scientific. Hydrogen peroxide

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(H_2O_2) was provided *gratis* by Solvay Interox. Double deionized water was purified to 18 $M\Omega\bullet cm$ with a Barnstead Nanopure II deionizing unit.

Probe Compounds. Based on a survey of rate constants for reactions with OH· and the potential for aerobic biodegradability of hundreds of potential probe compounds, two probes were selected based on their level of reactivity with OH· and potential for aerobic biodegradation: (1) tetrachloroethene (PCE), which reacts rapidly with hydroxyl radicals [$k_{OH·} = 2.6 \times 10^9 M^{-1} s^{-1}$ (*22*)], but is not biodegraded under aerobic conditions (*26*); and (2) oxalate (OA), which is characterized by negligible reactivity with OH· [$k_{OH·} = 7.7 \times 10^6 M^{-1} s^{-1}$ (*22*)], but can act as the primary carbon source for microbial metabolism. A concentration of 0.15 mM PCE was used in microbial-Fenton's experiments to minimize potential toxicity to the microorganisms. Oxalate stocks (32.5 mM) were prepared by dissolving OA in deionized water and adjusting to pH 7.0 with 1 N KOH. The OA solution was then mixed with the bacterial growth media. The maximum rate of cell growth, as measured by the maximum slope of optical density versus time, was achieved using 6.5 mM OA prior to conducting the abiotic−biotic reactions Therefore, an initial OA concentration of 6.5 mM was used in subsequent experiments.

Microorganism and Culture Conditions. *Xanthobacter flavus* strain FB71 was isolated by Büyüksönmez et al. (*23*) from activated sludge as a microorganism that can assimilate oxalate and utilize it as a sole carbon source. The defined growth medium consisted of M9 medium (3.4 g of Na_2HPO_4, 1.5 g of KH_2PO_4, 0.25 g of NaCl, 0.5 g of NH_4Cl, 0.12 g of $MgSO_4$, and 5.55 mg of $CaCl_2$ per L) (*24*), Wolfe's mineral solution (2 mL/L) (*25*), vitamin stock (2 mL/L of a stock containing 50 mg/L each biotin, thiamine HCl, and nicotinic acid), 6.5 mM OA, and 4 g/L nutrient broth to increase cell growth. After inoculation, 500 mL of media were stirred at 150 rpm in an incubator shaker at 35 °C for 3 days, resulting in a stock of *X. flavus* that was in stationary growth phase. The suspended biomass was then centrifuged at 10 000 g, and the cells were resuspended in a final 200 mL volume containing 0.1% strength M9 buffer for an approximate cell concentration of 10^9 CFU/mL, which was determined by enumeration on spread plates.

Modified Fenton's Reagent. Iron(III) was used as a catalyst to provide a superoxide-driven Fenton-like reaction (*7*). The iron(III) was chelated with NTA because of the ability of this complex to catalyze Fenton's reactions at the neutral pH required by many microorganisms, and because it provides a model of iron−organic matter complexes occurring in the subsurface. The iron(III) sulfate−NTA (1:1 ratio) catalyst was prepared using the procedure outlined by Pignatello and Baehr (*8*). NTA was dissolved in deionized water before an equimolar amount of ferric sulfate was added. The resulting chelated complex was then adjusted to pH 7.0 by 1 N KOH. Final Fe−NTA concentrations varied from 1 mM to 5 mM. Hydrogen peroxide was used in concentrations from 0.15 mM to 8.8 mM.

Experimental Procedures. All experiments were conducted in 40 mL borosilicate glass vials protected from light and loosely capped with PTFE-lined septa. Triplicate vials were established for each time point; temperature was maintained at 20 °C ± 2 °C. Hydrogen peroxide was injected at 10 min intervals to maintain a quasi-steady-state concentration, as bacterial catalase activity and catalysis by iron-NTA continually decomposed a portion of the H_2O_2. The H_2O_2 dosage required at each time of injection was determined empirically prior to the start of each experiment. H_2O_2 concentrations decreased no more than 5% prior to each reinjection. Vials were not shaken during incubation.

At selected time intervals, a triplicate set of vials was sacrificed and aliquots were collected for PCE and OA analysis.

PCE aliquots were extracted with hexane while the OA aliquots were filtered to remove the suspended biomass (using a Whatman 0.2 μm syringe filter) and then analyzed for OA concentration. Spread plates containing tryptic soy agar were used at each time point to check for potential microbial contamination.

Two sets of triplicate control reactions were conducted in parallel with PCE experiments, in which double deionized water replaced (1) hydrogen peroxide and (2) the Fe−NTA catalyst. No chloride release was observed in any of the controls. For OA control experiments, double deionized water was used in place of biomass.

Scavenging and Verification Studies. To confirm the reaction mechanisms present in the Fenton-like reaction conditions used in this study, 2-propanol and trichloromethane were used as oxidant and reductant scavengers, respectively. 2-Propanol [$k_{OH·} = 6 \times 10^9 M^{-1} s^{-1}$ (*22*)] and trichloromethane [$k_{e^-} = 3 \times 10^{10} M^{-1} s^{-1}$ (*22*)] were added to the systems at scavenger:probe molar ratios of 100:1 (*16*) to scavenge hydroxyl radicals or any reductants, such as superoxide anion or hydroperoxide anion, potentially present in the system (*17*). To verify oxidation of PCE, chloride ions were measured at selected time points in parallel to PCE oxidation. Separate reactions were conducted for chloride analysis and PCE analysis, and controls were conducted in parallel with deionized water in place of H_2O_2. Chloride standards were prepared by diluting a 0.1000 M ± 0.0005 M sodium chloride solution with deionized water. The reactivities of PCE and OA in the modified Fenton's systems were confirmed by conducting the reactions in the absence of microorganisms. The degree of microbial degradation of OA and lack of oxidation of PCE was verified by evaluating their loss in a microbial system without the addition of H_2O_2.

Experimental Design. Two-level central composite rotatable experimental designs were used to evaluate the effects of a series of process conditions on hydroxyl radical oxidations (quantified by PCE oxidation) and heterotrophic aerobic metabolism (measured by OA assimilation). Central composite experimental rotatable designs are used to analyze the interactive effects between variables and to produce a response surface illustrating interactions between variables (*27*). The central composite matrix was characterized by five center points and four star points set at a factor of 1.4142 on the far end of the coded scale to achieve complete rotatability (*28*).

The three variables studied in the loss of the probe compounds PCE and OA were Fe−NTA concentration (0−5 mM), H_2O_2 concentration (0.5−15 mM), and biomass level (10^5−10^9 CFU/mL). Two central composite designs were used to investigate OH·-mediated oxidations and *X. flavus* metabolism with varying Fe−NTA and H_2O_2 concentrations; the remaining two matrices were used to study the effect of cell mass and H_2O_2 concentration on OH·-mediated oxidations and heterotrophic metabolism (as measured by loss of the probe compounds). All reactions were conducted as described above in Experimental Procedures. Regression equations were developed using the least-squares method, and each term of the equation was evaluated within a 90% interval error of a single-sided t-distribution. Correlation (R^2) values were determined by comparing experimental values to values calculated using the regression equations. Contour isoconcentration lines for probe compound loss were developed using SYSTAT software.

Analysis. Hexane extracts containing PCE were analyzed using a Hewlett-Packard 5890A gas chromatograph with an electron capture detector and a 15 m × 0.32 DB-1 capillary column. GC parameters included an injector temperature of 240 °C, detector temperature of 350 °C, initial oven temperature of 40 °C, initial time of 2.0 min, program rate of 5 °C/min, and final temperature of 120 °C. Oxalate was
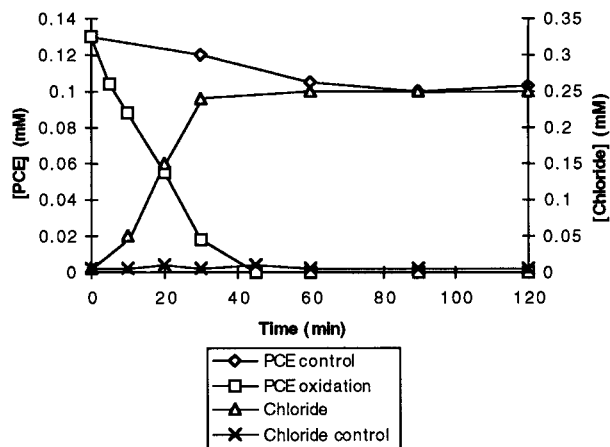
FIGURE 1. Fenton-like oxidation of PCE in relation to amount of chloride recovered (sample reactors: 0.13 mM PCE, 5 mM Fe−NTA, 1.5 mM quasi-steady-state H_2O_2; 10 mL total volume at pH 7.0; control reactors: H_2O_2 substituted by deionized water; $T = 20 \pm 2$ °C).

measured spectrophotometrically using a mixture of oxalate oxidase, 3-methyl-2-benzothiazolinine hydrazone, 3-(dimethylamino)benzoic acid, and peroxidase that yielded a purple indamine dye having an absorbance at 590 nm (*29–34*). Hydrogen peroxide concentration was determined by visible spectrophotometry after color development with $TiSO_4$ (*35*). Chloride ion concentrations were measured by a Fisher ion specific electrode and a double junction reference electrode with a Fisher Accumet pH meter 9100.

Results and Discussion

Reactivity of Probe Compounds in Modified Fenton's Reactions and in Microbial Systems. Before coexisting abiotic−biotic reactions were investigated, reactions were conducted to confirm (1) the existence of a hydroxyl radical mechanism in the Fenton-like system catalyzed by Fe−NTA, (2) minimal reactivity of OA with hydroxyl radicals, and (3) uptake of OA and negligible transformation of PCE through aerobic microbial metabolism. The oxidation of PCE using 5 mM Fe−NTA with a quasi-steady-state H_2O_2 concentration of 1.5 mM at pH 7.0 is shown in Figure 1. PCE was degraded to undetectable concentrations within 1 h, while PCE concentrations in control systems containing no H_2O_2 decreased by 20% due to volatilization. The chloride concentration increased steadily over 1 h, reaching a maximum ratio of 2.7 mol of Cl⁻ released per mole of PCE degraded with no increase in chloride concentration in the controls, confirming PCE oxidation. These results confirm previous findings that Fe−NTA is an effective catalyst for Fenton-like reactions at neutral pH (*8*).

PCE may be degraded via both oxidative and reductive pathways, and reducing species have recently been documented in modified Fenton's reactions (*17*). To determine whether reductants were involved in PCE oxidation in the Fe−NTA catalyzed Fenton's system, a set of parallel reactions was conducted using 2-propanol and trichloromethane as OH• and reductant scavengers, respectively. Addition of 2-propanol resulted in negligible PCE oxidation over 2 h in relation to control data (Figure 2), confirming that OH• is the primary reactant in PCE oxidation. Conversely, the data of Figure 2 show that destruction of PCE was not affected by the addition of excess trichloromethane, demonstrating that reducing species are not involved in PCE oxidation in this system. Oxalate, the probe compound used to monitor microbial metabolism, was evaluated for reactivity with OH•; negligible transformation of OA was observed over 2 h in the Fe−NTA catalyzed Fenton's system (Figure 2).
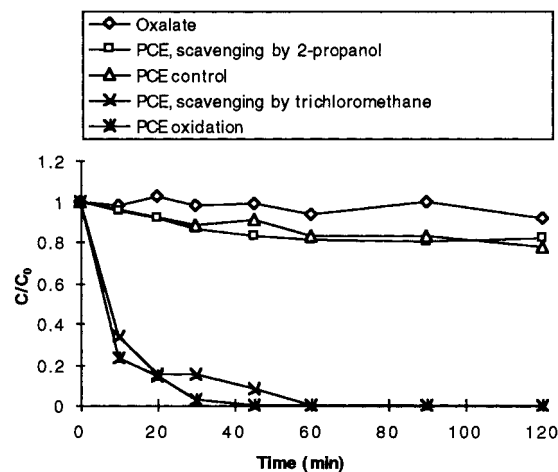

FIGURE 2. Fenton-like treatment of OA and PCE with and without scavengers (sample reactors: 0.15 mM PCE (0.03 mM PCE was used with scavenging by trichloromethane) or 6.5 mM OA, 1 mM Fe−NTA, 8.8 mM quasi-steady-state H_2O_2, 10 mL total volume at pH 7.0; control reactors: H_2O_2 substituted by deionized water; scavenger reactors: either 1 M 2-propanol or 8 mM trichloromethane; $T = 20 \pm 2$ °C).
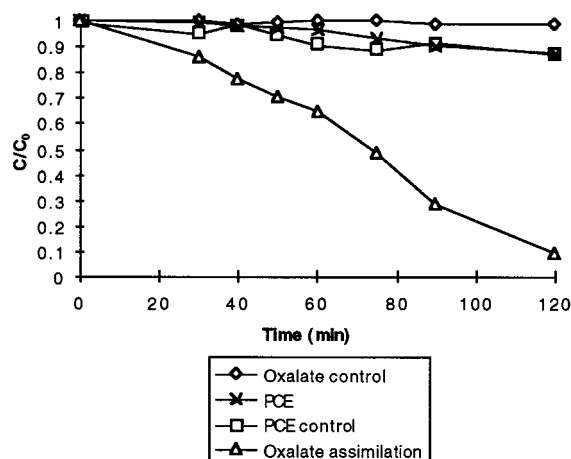

FIGURE 3. Effect of *Xanthobacter flavus* on PCE and OA in the absence of H_2O_2 (sample reactors: 0.15 mM PCE or 6.5 mM OA, 1 mM Fe−NTA, 10^{10} CFU/mL, 10 mL total volume at pH 7.0; control reactors: cells substituted by deionized water; $T = 20 \pm 2$ °C).

Although PCE biodegradation has been shown to occur only under anaerobic conditions (*26*), the oxidation of PCE by the aerobic microbe *X. flavus* was evaluated using cell cultures at a concentration of 10^{10} CFU/mL with 0.15 mM PCE and 6.5 mM OA in the absence of modified Fenton's reagent. Over 90% of OA was metabolized over 2 h, while PCE loss was ∼15% (Figure 3), which was similar to the volatilization loss observed in control samples; in addition, no chloride was detected in these cell cultures. These data confirm that PCE was not biodegraded by *X. flavus* under the conditions used.

Simultaneous Abiotic−Biotic Reactions in a Microbial-Fenton's System. Various conditions in modified Fenton's-microbial systems were evaluated to investigate the possible coexistence of chemical and microbial oxidations. In the first set of experiments (Figure 4), biomass and Fe−NTA catalyst concentrations were held constant at 10^9 CFU/mL and 1 mM, respectively, while the quasi-steady-state concentration of H_2O_2 was varied from 0.15 mM to 8.8 mM. At 0.15 mM H_2O_2, PCE was oxidized by 15% compared to control systems, while 95% of OA was assimilated by *X. flavus* (Figure 4a). At a quasi-steady-state H_2O_2 concentration of 0.74 mM, PCE oxidation increased to 25% (Figure 4b), while 95% of the OA
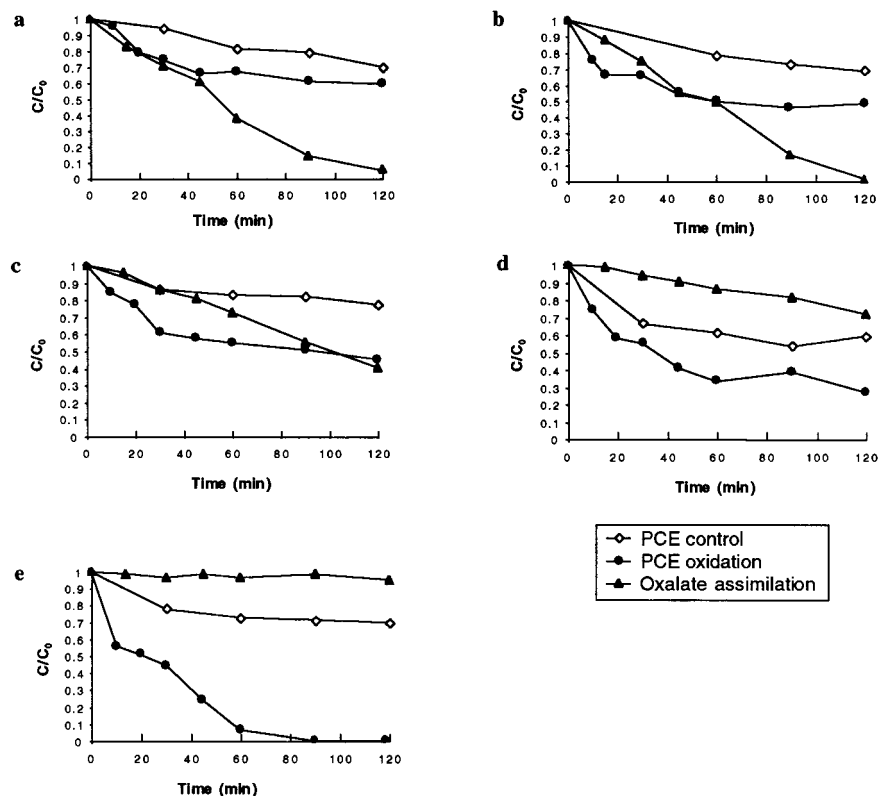
FIGURE 4. Coexisting abiotic—biotic reactions for PCE oxidation and OA assimilation (sample reactors: 0.15 mM PCE or 6.5 mM OA, 1 mM Fe—NTA, 10^9 CFU/mL, 10 mL total volume at pH 7.0; control reactors: H_2O_2 substituted by deionized water; $T = 20 \pm 2$ °C). Quasi-steady-state H_2O_2 concentration: (a) 0.15 mM; (b) 0.74 mM; (c) 1.5 mM; (d) 2.9 mM; and (e) 8.8 mM.

was metabolized during the same period. However, when the H_2O_2 concentration was increased to 1.5 mM and 2.9 mM, OA assimilation declined to 60% and 20%, respectively, accompanied by an increase in PCE destruction to 35% and 50%, respectively (Figure 4c,d). The trend of decreased OA assimilation continued when the H_2O_2 concentration was increased to 8.8 mM, where minimal OA assimilation and >99% PCE oxidation was observed (Figure 4e). Although hydrogen peroxide is toxic to microorganisms (4), it has been well documented that bacteria can remain metabolically active in its presence (5). However, an active role of OH$^\bullet$ in microbial systems containing high biomass levels (e.g., 10^9 CFU/mL) has not previously been reported. These results document that heterotrophic bacterial metabolism can occur in the presence of OH$^\bullet$ and that higher H_2O_2 concentrations favor OH$^\bullet$-mediated oxidations at the expense of bacterial metabolism.

Although hydroxyl radicals react with the biomass in these systems [OH$^\bullet$ reacts with most biological molecules at diffusion-controlled rates (22)], there is still a sufficient steady-state hydroxyl radical concentration to react with the probe PCE. Therefore, the results of Figure 4 suggest that when H_2O_2 is injected into the subsurface as a supply of O_2 during bioremediation, the Fenton-like reactions that also occur can coexist with the microbial processes. In addition, a recent trend in subsurface remediation is in situ chemical oxidation, in which H_2O_2 and catalysts are injected to promote Fenton-like oxidations. Büyüksonmez et al. (23) reported that microorganisms can survive in the presence of low fluxes of OH$^\bullet$ and grow significantly on the oxidation products of PCE. The results of Figure 4 suggest that, under some conditions, microbial metabolism might exist simultaneously with Fenton-like reactions during in situ chemical oxidation.

Evaluation of Maximum Occurrence of Simultaneous Abiotic—Biotic Oxidations. Two sets of two-level central composite rotatable design experiments were conducted for

each probe compound by (1) varying the Fe—NTA concentration (0—5 mM) and the H_2O_2 concentration (0.5—15 mM) while holding the *X. flavus* population constant at 10^{10} CFU/mL and (2) holding the Fe—NTA concentration constant at 5 mM while varying the *X. flavus* population (10^5—10^9 CFU/mL) and H_2O_2 concentration (0.5—15 mM).

Following the procedures outlined by Diamond (27), regression equations were developed from the experimental data to describe the loss of the probe compounds with varying Fe—NTA and H_2O_2 concentrations and constant biomass

$$\text{\% loss of PCE (OH}^\bullet \text{ production)} = 53 + \\ 6.31(\text{Fe} - 2.5) + 4.64(\text{H}_2\text{O}_2 - 7.35) - 2.12(\text{Fe} - 2.5)^2 - \\ 0.02(\text{H}_2\text{O}_2 - 7.35)^2 \quad (2)$$

$$\text{\% assimilation of OA (microbial metabolism)} = \\ 12.6 - 0.9(\text{Fe} - 2.5) - 5.5(\text{H}_2\text{O}_2 - 7.35) - \\ 0.48(\text{Fe} - 2.5)^2 + 0.6 \ (\text{H}_2\text{O}_2 - 7.35)^2 + 0.19(\text{Fe} - 2.5) \\ (\text{H}_2\text{O}_2 - 7.35) \quad (3)$$

where Fe = Fe—NTA concentration (mM) and H_2O_2 = hydrogen peroxide concentration (mM).

Equations 2 and 3 were characterized by an R^2 of 0.71 and 0.76, respectively, when values generated by these equations were compared to the experimental data, with every term being within the 90% confidence interval of a single-sided t-distribution. Response surfaces graphically depicting the equations are shown in Figure 5. Due to a larger initial biomass level in the central composite experiments, PCE and OA loss are not directly comparable to the results shown in Figure 4. However, similar to the data shown in Figure 4, higher concentrations of H_2O_2 resulted in increased generation of OH$^\bullet$ and lower levels of microbial metabolism. Greater than 80% oxidation of PCE was achieved within 2 h
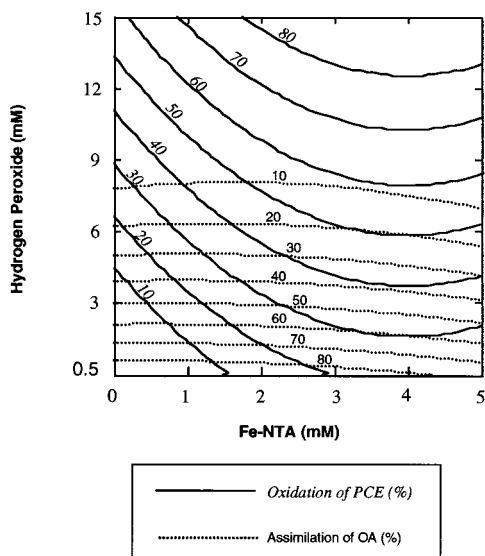
FIGURE 5. Response surfaces for PCE oxidation and OA assimilation as a function of Fe−NTA and H_2O_2 (sample reactors: 0.15 mM PCE or 6.5 mM OA, 0−5 mM Fe−NTA, 0.5−15 mM quasi-steady-state H_2O_2; 10 mL total volume at pH 7.0, 10^{10} CFU/mL; control reactors: H_2O_2 substituted by deionized water; $T = 20 \pm 2\ ^\circ C$).

with 5 mM Fe−NTA and 15 mM H_2O_2 (solid lines). Under these same conditions, minimal OA assimilation was achieved (dotted lines). In contrast, >80% of the OA was metabolized at H_2O_2 concentrations below 0.74 mM regardless of the Fe−NTA concentration, while PCE oxidation ranged from 10%−30%, depending on the Fe−NTA concentration. Inspection of Figure 5 also shows that near-equal coexisting abiotic−biotic reactions occurred at 4.4 mM H_2O_2 and 2.5 mM Fe−NTA, with 35% loss of both probe compounds.

Equations 4 and 5, representing the loss of the probe compounds with varying H_2O_2 concentration and *X. flavus* population and constant Fe−NTA concentration, were developed from the second set of central composite experiments

$$\% \text{ loss of PCE (OH}^\bullet \text{ production)} = 42 + 5.51(H_2O_2 - 7.35) - 1.09 \times 10^{-8}(\text{cells} - 5 \times 10^8) + 0.08(H_2O_2 - 7.35)^2 + 6.92 \times 10^{-17}(\text{cells} - 5 \times 10^8)^2 \quad (4)$$

$$\% \text{ assimilation of OA (microbial metabolism)} = 8.2 - 4.22(H_2O_2 - 7.35) + 7.66 \times 10^{-9}(\text{cells} - 5 \times 10^8) + 0.58(H_2O_2 - 7.35)^2 - 3.92 \times 10^{-17}(\text{cells} - 5 \times 10^8)^2 - 4.77 \times 10^{-10}(H_2O_2 - 7.35)(\text{cells} - 5 \times 10^8) \quad (5)$$

where H_2O_2 = hydrogen peroxide concentration (mM) and cells = cell mass (CFU/mL).

Equations 4 and 5 were characterized by an R^2 of 0.95 and 0.80, respectively, when values generated by these equations were compared to the experimental data, with all terms being within the 90% confidence interval of a single-sided t-distribution. Response surfaces generated by eqs 4 and 5, illustrating the interactive effects of biomass and H_2O_2 concentrations, are shown in Figure 6. Solid contour lines in Figure 6 illustrate a decrease in PCE oxidation with increasing cell mass, which may be due to the increased catalase activity associated with the higher biomass (22). Conversely, increasing H_2O_2 concentrations led to greater generation of OH$^\bullet$ and reduced uptake of OA (dotted lines). Greater than 90% PCE oxidation was observed at H_2O_2 levels above 12 mM and cell mass below 10^6 CFU/mL, while OA assimilation was minimal under those conditions. OA as-
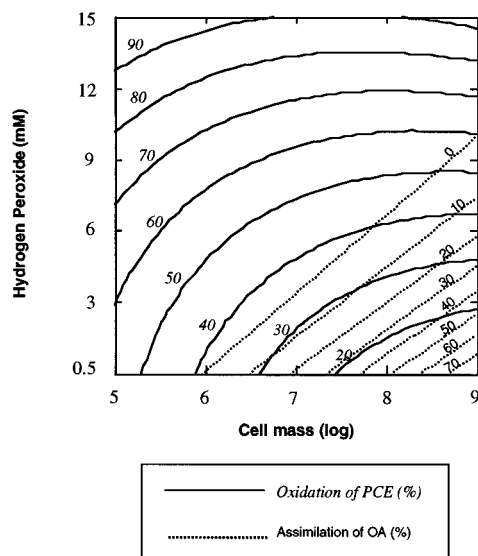

FIGURE 6. Response surfaces for PCE oxidation and OA assimilation as a function of H_2O_2 and cell mass (sample reactors: 0.15 mM PCE or 6.5 mM OA, 10^5-10^9 CFU/mL, 0.5−15 mM quasi-steady-state H_2O_2, 10 mL total volume at pH 7.0, 5 mM Fe−NTA; control reactors: H_2O_2 substituted by deionized water; $T = 20 \pm 2\ ^\circ C$).

similation of 70% was achieved at 10^9 CFU/mL and <1 mM H_2O_2 over 2 h. However, no OA assimilation was noted with a cell mass below 10^6 CFU/mL even in the absence of H_2O_2, which may have been due to insufficient time for the low biomass level to assimilate significant amounts of OA. Approximately equal degrees of simultaneous abiotic−biotic oxidation of PCE and assimilation of OA were achieved at 10^9 CFU/mL and 4.6 mM H_2O_2, with 30% loss of both probe compounds.

The most common target concentration for stabilized H_2O_2 injected into the subsurface has been 3 mM (100 mg/L), which was selected because it generally resulted in minimal toxicity to microorganisms (4). The data of Figure 4 show that, in the presence of chelated iron (e.g., an iron−organic matter complex), 3 mM H_2O_2 also promotes the generation of OH$^\bullet$, even at high biomass concentrations. As a result, OH$^\bullet$ may have been generated in subsurface systems in which aerobic bioremediation was promoted by the injection of stabilized H_2O_2. The catalysis of H_2O_2 in the subsurface is highly complex, with possible initiation of Fenton-like reactions by soluble iron, chelated iron (7, 8), and metal oxyhydroxides (9−14). The solubility of chelated iron at neutral pH makes it an effective Fenton's catalyst in biological systems. Iron oxyhydroxides promote Fenton-like reactions by oxidation−reduction reactions on the oxide surface, which occur at both neutral and acidic pH (36). Therefore, a variety of mechanisms may promote Fenton-like oxidations during the injection of H_2O_2 in the subsurface for bioremediation.

Although relatively high concentrations of H_2O_2 are used for the in situ chemical oxidation of contaminants in the subsurface, the H_2O_2 decomposes rapidly and concentrations below 5 mM are commonly found in these systems. Recent results have shown that bacteria can survive high concentrations of H_2O_2 in the subsurface and then grow rapidly on the partially oxidized degradation products of the contaminants (37). Therefore, the potential exists for significant bacterial metabolism in the presence of Fenton-like reactions driven by a residual concentration of 2−5 mM H_2O_2. The evidence of coexisting abiotic−biotic reactions in microbial-Fenton's systems provided by this research suggests that the optimum process design for in situ bioremediation using H_2O_2 as an oxygen source or for in situ chemical oxidation through

modified Fenton's reagent should incorporate the potential for these coexisting reactions, which could promote enhanced treatment and contaminant mineralization. Furthermore, these coexisting oxidations may provide the basis for novel reactor systems for the ex situ treatment of recycle water, cooling water, and industrial wastewaters. A hybrid system may have numerous advantages over present chemical-biological treatment systems (*36, 39*) because it eliminates the need for oxidative pretreatment prior to biological treatment.

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